ENCLOSURE 3

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 January 2005 (06.01.2005)

PCT

(10) International Publication Number
WO 2005/000321 A1

(51) International Patent Classification[7]: A61K 31/715, A61P 17/02

(21) International Application Number: PCT/EP2004/051209

(22) International Filing Date: 23 June 2004 (23.06.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
MI2003A001291 25 June 2003 (25.06.2003) IT

(71) Applicant *(for all designated States except US)*: RICERFARMA S.r.l. [IT/IT]; Via Egadi 7, I-20144 MILAN (IT).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: MACCHI, Franco [IT/IT]; Via Caravaggio 14, I-21049 TRADATE (IT).

(74) Agent: GERVASI, Gemma; NOTARBARTOLO & GERVASI S.p.A., Corso di Porta Vittoria 9, I-20122 MILAN (IT).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/000321 A1

(54) Title: USE OF HYALURONIC ACID FOR PREPARING COMPOSITIONS FOR TREATING ORAL CAVITY APHTHAS

(57) Abstract: Use of hyaluronic acid as the sole active ingredient for preparing compositions in particular for topical use for treating oral cavity aphthas.

USE OF HYALURONIC ACID FOR PREPARING COMPOSITIONS FOR TREATING ORAL CAVITY APHTHAS

FIELD OF THE INVENTION

The present invention relates to the use of hyaluronic acid for treating oral cavity aphthas.

STATE OF THE ART

Aphthas, better known as recurrent oral aphthous ulcerations (ROAU), are ulcerous pathologies of the oral mucosa which affect more than 20% of the population. The etiology of this ailment is yet to be defined. Aphthas are round or oval protuberant ulcers, surrounded by bright red areolas, on the smooth tissue of the mucosa. Almost all types of aphthas, including small ones, are capable of causing pain.

Of the susceptible individuals one in ten will have monthly episodes, whereas the majority have 3-4 episodes of new lesions per year occur. Untreated lesions in general last for 7-10 days and heal without leaving scars. In general, aphtha treatments are intended to ease symptoms, although many types of therapies for treating aphthas have been considered.

For example analgesics for topical use have been employed for relieving symptoms, and anti-inflammatories for reducing pathological changes, while anti-bacterials have been contemplated for controlling microbial contaminations and secondary infections.

Anti-bacterial agents include antibiotics (tetracycline) and antiseptics (clorhexidine).

Mouthwashes containing wide spectrum antibiotics have been able to reduce new ulcers, following a 10 day treatment. This effect is due to a reduced oral microflora thereby reducing the effects of a secondary infection.

However, antibiotics have a potentially undesirable mycotic effect and can give rise to allergic reactions.

Anti-bacterial mouthwashes can provide some benefit by controlling pain, reducing both the effects caused by a secondary infection and the duration of the ulcer. Clorhexidine can reduce the total number of days with ulceration, but has

not been at all effective on the incidence or severity thereof. Furthermore, it frequently gives rise to colour changes on the teeth and tongue and upsets taste sensation.

Hyaluronic acid is a natural constituent of connective tissue.

EP-A1-0444492 describes the topical use of high molecular weight hyaluronic acid for treating inflammatory diseases of the oral cavity, such as gingivitis.

WO 0209637 discloses pharmaceutical compositions for the topical treatment of inflammatory diseases of the oral mucosa such as stomatitis, containing an association of hyaluronic acid, glycyrrhetinic acid and polyvinylpyrrolidone

SUMMARY OF THE INVENTION

The Applicant has found that hyaluronic acid is able to effectively cure oral cavity aphthas.

In this respect, the Applicant has surprisingly found that hyaluronic acid is not only able to alleviate the symptoms and reduce the duration of ulceration, as well as the severity thereof.

An aspect of the present invention is therefore the use of hyaluronic acid for preparing compositions, in particular for topical use, for treating oral cavity aphthas.

DETAILED DESCRIPTION OF THE INVENTION

The compositions containing hyaluronic acid for use in accordance with the invention are preferably liquid, solid and/or semisolid preparations in the form of O/W (oil in water) and W/O (water in oil) emulsions, ointments and creams, pastes, gels, solutions, suspensions, dispersions, powders, tensiolytes, oleolytes, or any other rheological form suitable for use alone or in combination with the other forms, also in the form of tablets, pills, gums, or in the form of any other applicative solutions known in the art and suitable for topical use in the oral cavity.

Even more preferably, the topical compositions for use in accordance with the present invention are in the form of oral cavity gels, mouthwashes and sprays.

Preferably hyaluronic acid is in the form of the sodium salt. Hyaluronic acid has preferably a molecular weight of between 800,000 and 4,000,000, even more

preferably between 1,000,000 and 2,000,000.

The topical compositions of the present invention preferably contain hyaluronic acid in the form of the sodium salt at concentrations of between 0.01 and 10% by weight on the total weight of the composition, more preferably between 0.01 and 5% by weight.

Some illustrative but non-limiting examples of compositions for topical use based on sodium hyaluronate are given.

Composition 1: gel

Sodium hyaluronate average molecular weight 1,500,000:	0.240 w/w
Xylitol	7.500 w/w
Sodium carboxymethylcellulose	4.500 w/w
PEG 40 hydrogenated castor oil	1.000 w/w
Glyceryl monolaurate	0.700 w/w
Polycarbophil	0.800 w/w
Lactic acid (Pharm.)	0.060 w/w
Sodium lactate	0.100 w/w
EDTA	0.050 w/w
Sodium saccharinate	0.220 w/w
Flavour	0.500 w/w
Dichlorobenzylalcohol	0.500 w/w
Colorant CI 42090 (FD&C BLUE 1)	0.00012 w/w
Colorant CI 47005 (D&C YELLOW 10)	0.00028 w/w
Sodium hydroxide	to pH=6.5
Water	remainder to 100

Composition 2: mouthwash

Sodium hyaluronate average molecular weight 1,500,000:	0.025 w/w
Xylitol	7.500 w/w
PEG 40 hydrogenated castor oil	0.600 w/w
Polycarbophil	0.150 w/w
Lactic acid (Pharm.)	0.060 w/w
Sodium lactate	0.100 w/w

EDTA	0.050 w/w
Sodium saccharinate	0.018 w/w
Flavour	0.100 w/w
Dichlorobenzylalcohol	0.500 w/w
Polysorbate 20	0.800 w/w
Colorant CI 42090 (FD&C BLUE 1)	0.00012 w/w
Colorant CI 47005 (D&C YELLOW 10)	0.00028 w/w
Sodium hydroxide	to pH=6.5
Demineralized Water	remainder to 100

Composition 3: spray

Sodium hyaluronate	0.100 w/w
Xylitol	7.500 w/w
PEG 40 hydrogenated castor oil	0.500 w/w
Dichlorobenzylalcohol	0.500 w/w
Lactic acid (Pharm.)	0.060 w/w
Sodium lactate	0.100 w/w
EDTA	0.050 w/w
Sodium saccharinate	0.220 w/w
Flavour	0.200 w/w
PVA	0.050 w/w
Propylene glycol	4.000 w/w
Sodium hydroxide	to pH=6.5
Demineralized water	remainder to 100

CLINICAL STUDY

A) STUDY DESIGN

This controlled study used a double blind, single centre, parallel group design to determine the efficacy of a gel formulation in relieving the symptoms in subjects with recurrent oral aphthous ulceration.

B) STUDY POPULATION

B1) Number of Subjects

The investigator enrolled a sufficient number of subjects in the study to achieve a study population of 120 evaluable subjects (60 in each group) with ROAU.

B2) Subject-Selection Criteria

Inclusion Criteria To be eligible for study partecipation the subject had to meet the following criteria:

- The subject must be between 18 and 65 years of age
- A history of ROAU > 2 times per year
- Current aphthous ulcer/ulcers present for < 3 day

B3) Exclusion Criteria

Any of the following conditions excluded subjects from eligibility for study partecipation:

- Patients with underlying white blood cell disorder
- Patients taking systemic hemotherapy, immunosuppressants, or who sufer from drug-related recurrent aphthous ulceration
- Patients suffering from malignant disease
- Patients with uncorrected dietary defect
- Pregnant or breast feeding women
- A history of sensitivity of mouthwashes

B4) Prohibited/Allowable Medications

Prohibited Medications

- Any topical or systemic treatment for ROAU including steroids and vitamins B1 and B6 other than study treatments
- Antiseptic mouthwashes
- Systemic chemotherapy, immunosuppressants
- Rx or OTC nonsteroidal anti-inflammatory drugs including, but not limited to aspirin, diclofenac, diflunisal, etodolac, ibuprofen, indomethacin, ketoprofen, ketorolac, nabumetone, naproxen, naproxen sodium, oxaprozin, piroxicam, or sulindac.

Allowable Medications

- Any medication not specifically prohybited
- Paracetamol

C) STUDY METHODOLOGY

Subjects were recruited from an existing group of patients with ROAU who have been screened for known causative factors or when they present as new patients to the clinic. Existing patients or patients attending screening who do not have a current ulcer will be asked to contact the clinic at the time of onset of their next aphthous ulcer.

C1) Clinic Visit 1 (Day 1): Screened subjects meeting the selection criteria for the study described had the study explained to them and if they agreed to partecipate signed an informed consent form. They will be allocated a subsequential subject number. The subjects demographic history and history of ROAU were recorded together with details of their current episode of aphthous ulceration including the time and date of onset, number, size and position of mouth ulcers.

The study nurse explained to the subjects how to fill out the 10 cm visual analogue scale (VAS) used to score their level of discomfort or soreness arising from their mouth ulcer. Subjects recorded their discomfort from their ulcer prior to gel application (baseline). They applied the gel to the ulcerated area under supervision with 1-2 ml of their assigned gel having one of the following two composition:

Product name	Ingredients
Hyaluronic Acid Gel 0.2%	Aqua, xylitol, cellulose gum, alcohol, PEG-40, Hydrogenated castor oil, sodium hyaluronate, Polyvinyl alcohol,polycarbophil, Dichlorobenzyl alcohol, aroma flavouring CI 40290
Placebo	Aqua, xylitol, cellulose gum, alcohol, PEG-40, Hydrogenated castor oil, Polyvinyl alcohol, polycarbophil, Diclorobenzyl alcohol, aroma flavouring

Subjects recorded their discomfort immediately after application and at 5, 10, 15, 20, 30, 45 and 60 minutes. The time of gel application will be recorded in the CRF and the subjects log diary. A stopwatch was used to record time measurements.

The subject will be supplied with sufficient tubes of the gel to take home. The study nurse instructed the subject how to fill out a log diary. The subject continued to record their VAS scores in their log diary at 2, 3 and 4 hours postgel application. The subjects will apply the gel again after their evening meal and record their VAS score 1 hour post application.

Appendix A; Time table of Visits and Procedures

C2) Day 2-7

Subjects continued to apply the gel at home 2 to 3 time daily, after breakfast and after their evening meal (and 1 other time during the day, if desired) from days 2-7, even if their ulcer has healed. VAS scores was recorded in the subjects' log diaries 1 hour post application in the morning and evening. Subjects recorded the severity of their mouth ulcers, any unpleasant effects of their study treatment and the severity of their mouth ulcers. Any new ulcers occurring was recorded in their log diaries.

C3) Clinic Visit 2 (Day 8):

Subjects returned to the clinic to review their completed log diaries with the study nurse and return remaining study material. They were asked to score their overall assessment of the gel on a 5 point scale. Subjects will be questioned about the occurrence of any adverse events.

Information obtained relating to adverse events were recorded on the associated pages of the CRF. The size, number and position of lesions present on day 8 were recorded on the CRF.

The VAS entries on each subjects log diary were measured and transcribed to the associated pages of the CRF.

C4) Efficacy Assessments

Following entry to the study, the study nurse recorded the subject's demographic details and examined the subject to determine the size, number and position of ulcers and record time of onset of ulcer.

C5) Primary Efficacy Parameter

Subjects recorded their discomfort/soreness scores on a 10 cm visual analogue scale (VAS).

The boundaries of the scales were "worst possible" and "no soreness".

Scores were completed at baseline and at 0, 5, 10, 15, 20, 30, 45 and 60 minutes post initial application. The gel application and completion of scores was done under supervised conditions in the Clinic. At the end of 60 minutes, the subjects continued to apply the gel at home 2 to 3 times daily and they were asked to record discomfort/soreness on the same VAS twice daily an hour after the morning and evening applications.

Two parameters were extrapolated from the serial VAS completed in the first hour:

a) Time in minutes to the maximum reduction in discomfort/soreness following dosing with the gel.

b) Serial VAS recorded in the first hour was compiled into a graph of discomfort soreness (mm) versus time (minutes). The area under the graph was measured using the trapezoidal method and recorded as AUC (0-60 minutes). This provided an overall assessment of each subjects discomfort/soreness experience throughout the initial observation period.

C6) Secondary Efficacy Parameter

At the end of the 7 day investigation period, subjects were asked if they have had any ulcer free days and their overall assessment of the gel based on the following scale:

Very good Good Moderate Poor Very Poor

D) RESULTS

In this randomized blind clinical study it was evidenced that if compared to placebo composition the gel composition containing hyaluronic acid proved able to reduce significantly the number of ulcers already in the fifth day, and also evidenced an overall beneficial effect in every investigated ROAU symtphomatology.

CLAIMS

1. Use of hyaluronic acid for preparing compositions for the treatment of oral cavity aphthas, wherein hyaluronic acid is the sole active ingredient.

2. Use as claimed in claim 1 wherein the hyaluronic acid is in the form of sodium salt.

3. Use as claimed in claim 2 wherein said compositions are suitable for topical application.

4. Use as claimed in claim 3 wherein said compositions for topical use contain sodium hyaluronate in concentrations between 0.01 and 10% by weight on the total weight of the composition.

5. Use as claimed in claim 4, characterised in that said concentration is between 0.01 and 5% by weight on the total weight of the composition.

6. Use as claimed in any one of claims 1-5, characterised in that said average molecular weight is between 800,000 and 4,000,000.

7. Use as claimed in any one of claims 1-5, wherein said average molecular weight of the hyaluronic acid is between 1,000,000 and 2,000,000.

INTERNATIONAL SEARCH REPORT

national Application No
T/EP2004/051209

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/715 A61P17/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 972 906 A (FALK RUDOLF EDGAR ET AL) 26 October 1999 (1999-10-26) page 1, paragraph 2 - paragraph 3 column 2, line 26 - line 40 column 5, line 35 - line 50	1-5
X	US 2002/183278 A1 (BRAGUTI GIANLUCA ET AL) 5 December 2002 (2002-12-05) paragraph '0002! paragraph '0017! claims 1,54,55	1-7

-/--

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 October 2004	02/11/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax (+31–70) 340–3016	Bonzano, C

2

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

ational Application No
/EP2004/051209

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RUSSELL A L: "PARALLELISM BETWEEN CUTANEOUS AND MUCOSAL PATHOLOGY. A NEW TEST BEDFOR AT 2101 (3% DICLOFENAC ACID IN 2.5% HYALURONAN)" ROYAL SOCIETY OF CHEMISTRY. ROUND TABLE SERIES, ROYAL SOCIETY OF MEDICINE SERVICES, LONDON, GB, vol. 40, 1 December 1995 (1995-12-01), pages 125-131, XP000603132 ISSN: 0268-3091 page 125, paragraph 2 - paragraph 6 page 129, paragraph 2	1-5

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

ıtional Application No
/EP2004/051209

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5972906 A	26-10-1999	US 5639738 A	17-06-1997
		US 6103704 A	15-08-2000
		US 5792753 A	11-08-1998
		US 5910489 A	08-06-1999
		WO 9407505 A1	14-04-1994
		WO 9526193 A1	05-10-1995
		WO 9529683 A1	09-11-1995
		WO 9530423 A2	16-11-1995
		WO 9606622 A1	07-03-1996
		WO 9817320 A1	30-04-1998
		EP 0952855 A1	03-11-1999
		US 5834444 A	10-11-1998
		US 5614506 A	25-03-1997
		US 5827834 A	27-10-1998
		US 6022866 A	08-02-2000
		US 5990095 A	23-11-1999
		US 6194392 B1	27-02-2001
		US 5852002 A	22-12-1998
		US 5830882 A	03-11-1998
		US 5817642 A	06-10-1998
		US 5811410 A	22-09-1998
		US 6017900 A	25-01-2000
		US 5962433 A	05-10-1999
		US 5977088 A	02-11-1999
		US 5824658 A	20-10-1998
		US 6087344 A	11-07-2000
		US 5817644 A	06-10-1998
		US 2004019011 A1	29-01-2004
		US 6475795 B1	05-11-2002
		US 2002077314 A1	20-06-2002
		US 6114314 A	05-09-2000
		US 5990096 A	23-11-1999
		US 5942498 A	24-08-1999
		US 6218373 B1	17-04-2001
		US 6147059 A	14-11-2000
		US 5914322 A	22-06-1999
		US 6136793 A	24-10-2000
US 2002183278 A1	05-12-2002	IT MI20001732 A1	28-01-2002
		AU 1211302 A	13-02-2002
		BR 0112962 A	24-06-2003
		CA 2424346 A1	07-02-2002
		CN 1474700 T	11-02-2004
		WO 0209637 A2	07-02-2002
		EP 1313489 A2	28-05-2003
		HR 20030046 A2	30-04-2004
		JP 2004505028 T	19-02-2004
		NO 20030411 A	27-01-2003
		NZ 523832 A	26-09-2003
		US 2002173485 A1	21-11-2002
		ZA 200300712 A	09-02-2004

Form PCT/ISA/210 (patent family annex) (January 2004)